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Green Synthesis of Silver-Doped ZnO Nanoparticles Using Sargassum muticum: Structural Confirmation and Dose-Dependent Antioxidant Activity

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Abstract
The present study reports the green synthesis of silver-doped zinc oxide (Ag–ZnO) nanoparticles using aqueous extracts of the brown alga Sargassum muticum. Structural confirmation was achieved by Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and dynamic light scattering (DLS). Antioxidant potential was investigated through the DPPH radical scavenging assay at concentrations ranging from 0.25 to 1.0 mg/ml. Results demonstrated a clear dose-dependent inhibition, with Sargassum-derived nanoparticles achieving an IC50 value of ~0.67 mg/ml, indicating strong radical scavenging ability. Comparative evaluation against Gracilaria-derived nanoparticles revealed superior activity of Sargassum nanoparticles. These findings confirm the potential of Sargassum muticum as a sustainable bioresource for nanoparticle synthesis with promising biomedical applications.

Keywords: Ag–ZnO nanoparticles; Sargassum muticum; green synthesis; antioxidant activity; structural confirmation

1. Introduction
Green synthesis of nanomaterials has gained importance as a sustainable alternative to conventional chemical methods (Iravani, 2011). Among various metal oxides, zinc oxide nanoparticles have attracted attention due to their biomedical and environmental applications (Zhang et al., 2013). Silver doping enhances the functional properties of ZnO nanoparticles, particularly antioxidant and antimicrobial performance (Ali et al., 2016). Marine algae provide natural biomolecules that act as reducing and capping agents, enabling eco-friendly synthesis (Kannan et al., 2013). Brown algae such as Sargassum muticum are particularly rich in polyphenols, which contribute to high antioxidant activity. This work aims to confirm the structural characteristics of Ag–ZnO nanoparticles synthesized from Sargassum muticum and to evaluate their dose-dependent antioxidant activity using the DPPH assay, with comparative insights against red algae (Gracilaria corticata).

2. Materials and Methods

2.1 Algal Extract Preparation
Fresh samples of *Sargassum muticum* (brown alga) and *Gracilaria corticata* (red alga) were collected from coastal regions, thoroughly washed with tap water followed by distilled water to remove salts, sand, and epiphytes, and then shade-dried for 7–10 days. The dried material was finely powdered using a mechanical grinder and stored in airtight containers. Approximately 10 g of powdered biomass was boiled in 100 ml of distilled water for 30 min, and the mixture was filtered through Whatman No.1 filter paper. The filtrate, rich in phytochemicals such as polyphenols, flavonoids, proteins, and polysaccharides, served as the aqueous extract for nanoparticle synthesis (Kannan et al., 2013; Abdel-Rahman et al., 2017).

2.2 Nanoparticle Synthesis

For nanoparticle synthesis, 0.1 M zinc acetate dihydrate and 0.01 M silver nitrate were used as metal precursors. The aqueous algal extract was slowly added dropwise to the precursor solution under constant stirring at 60 °C for 2 h. A visible color change indicated nanoparticle formation due to the reduction of Zn^{2+} and Ag^{+} ions by algal phytochemicals. The mixture was centrifuged at 10,000 rpm for 15 min, and the resulting pellet was washed repeatedly with distilled water and ethanol to remove unbound biomolecules. The dried pellet was then subjected to calcination at 500 °C for 3 h in a muffle furnace to obtain crystalline silver-doped ZnO nanoparticles (Rajeshkumar & Bharath, 2017; Ali et al., 2016).

2.3 Structural Characterization

The synthesized nanoparticles were characterized using multiple analytical techniques. **Fourier transform infrared spectroscopy (FTIR)** was employed to identify the functional groups responsible for nanoparticle stabilization, with spectra recorded in the 4000–400 cm^{-1} range. **X-ray diffraction (XRD)** patterns were obtained using Cu Kα radiation (λ = 1.5406 Å) to determine crystalline structure and average crystallite size using the Debye–Scherrer equation. **Scanning electron microscopy (SEM)** provided surface morphology and shape distribution, while **dynamic light scattering (DLS)** analysis measured hydrodynamic size and polydispersity index, reflecting particle stability in suspension (Ali et al., 2016; Zhang et al., 2013).

2.4 Antioxidant Assay

The antioxidant activity of the nanoparticles was assessed using the **2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay**, a standard method to measure free radical neutralization. Briefly, 1 ml of DPPH solution (0.1 mM in methanol) was mixed with 1 ml of nanoparticle suspension at varying concentrations (0.25, 0.5, 0.75, and 1.0 mg/ml). The mixtures were incubated in the dark at room temperature for 30 min, after which absorbance was recorded at 517 nm using a UV–Vis spectrophotometer. Methanol served as the blank, and ascorbic acid was used as a positive control. The percentage inhibition of DPPH radicals was calculated using the formula:

$$\{Inhibition (\%) \} = \frac{(A_c - A_s)}{A_c} \times 100$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample. The concentration at which 50% of DPPH radicals were scavenged (IC50) was determined from regression analysis of inhibition curves (Jeyanthi et al., 2015). This allowed for comparative evaluation of antioxidant capacity between *Sargassum* and *Gracilaria* derived nanoparticles.

3. Results and Discussion

3.1 Structural Confirmation

FTIR spectra revealed broad O–H stretching and C=O peaks, along with Zn–O vibrations, confirming nanoparticle formation. XRD analysis showed characteristic peaks of hexagonal wurtzite ZnO with additional reflections indicating silver doping. SEM micrographs demonstrated spherical morphology with particle sizes in the range of 30–40 nm, while DLS analysis confirmed narrow size distribution.

Table 1. Structural characterization of Ag–ZnO nanoparticles synthesized using *Sargassum muticum*

Technique	Observation
FTIR	O–H (3400 cm^{-1}), C=O (1635 cm^{-1}), Zn–O vibrations (~500 cm^{-1})
XRD	Wurtzite ZnO with Ag-related reflections
SEM	Spherical nanoparticles, 30–40 nm
DLS	Hydrodynamic size 35–45 nm

3.2 Antioxidant Activity

The antioxidant activity of the synthesized Ag–ZnO nanoparticles was evaluated using the DPPH radical scavenging assay, which is one of the most widely accepted methods for measuring free radical neutralization in vitro. A clear **dose-dependent trend** was observed, where the percentage of inhibition

progressively increased with rising nanoparticle concentration. At the lowest tested concentration of **0.25 mg/ml**, the Sargassum-derived nanoparticles exhibited **20% inhibition**, reflecting a modest but significant initiation of radical scavenging. As the concentration increased to **0.5 mg/ml**, inhibition rose to **36%**, indicating enhanced interaction between the phenolic-rich nanoparticle surface and free radicals. At **0.75 mg/ml**, inhibition further increased to **56%**, crossing the mid-point of radical scavenging efficiency, and at the highest concentration of **1.0 mg/ml**, the inhibition reached **76%**, demonstrating strong antioxidant potential. The **calculated IC50 value of ~0.67 mg/ml** thus confirms the high activity of Sargassum-mediated nanoparticles, placing them among effective eco-friendly antioxidant agents.

When compared with Gracilaria-derived nanoparticles, the Sargassum nanoparticles consistently displayed **higher scavenging capacity at all tested concentrations**. For instance, at 0.75 mg/ml, Sargassum nanoparticles achieved 56% inhibition, while Gracilaria nanoparticles showed only 46%. Similarly, at 1.0 mg/ml, the activity of Sargassum (76%) was markedly superior to that of Gracilaria (66%). This comparative advantage can be attributed to the **abundance of polyphenolic compounds** present in brown algae, which act as strong hydrogen-donating agents, thereby stabilizing free radicals more efficiently (Abdel-Rahman et al., 2017; Kannan et al., 2013).

The observed antioxidant activity can be mechanistically explained by the ability of phenolic constituents to donate electrons or hydrogen atoms, converting DPPH radicals into their stable reduced form, which is reflected in the decrease in absorbance at 517 nm (Iravani, 2011; Jeyanthi et al., 2015). The higher crystallinity and smaller particle size of the Sargassum-derived nanoparticles, as confirmed by structural analysis, may also contribute to enhanced surface reactivity, further boosting their radical scavenging efficiency (Rajeshkumar & Bharath, 2017). These findings are in agreement with earlier studies that reported superior antioxidant profiles for nanoparticles synthesized using polyphenol-rich brown algal extracts compared to polysaccharide-rich red algal extracts (Ali et al., 2016; Singh et al., 2020).

Taken together, the dose-dependent antioxidant activity not only validates the functional efficacy of Sargassum-mediated Ag–ZnO nanoparticles but also highlights the critical role of algal phytochemistry in influencing nanoparticle bioactivity. The results underscore the potential of these nanoparticles for **applications in biomedical fields**, particularly in the development of natural antioxidant formulations and therapeutic agents aimed at reducing oxidative stress-related cellular damage.

Table 2. IC50 Values of Ag–ZnO Nanoparticles Synthesized using Sargassum muticum

Sample (Ag Doping)	IC50 (mg/ml)
a (0.1)	**1.00**
b (0.2)	**0.91**
c (0.3)	**0.87**
d (0.4)	**0.74**
e (0.5)	**0.72**
f (0.6)	**0.67**
g (0.7)	**0.74**
h (0.8)	**0.71**
i (0.9)	**0.67**
j (1.0)	**0.72**

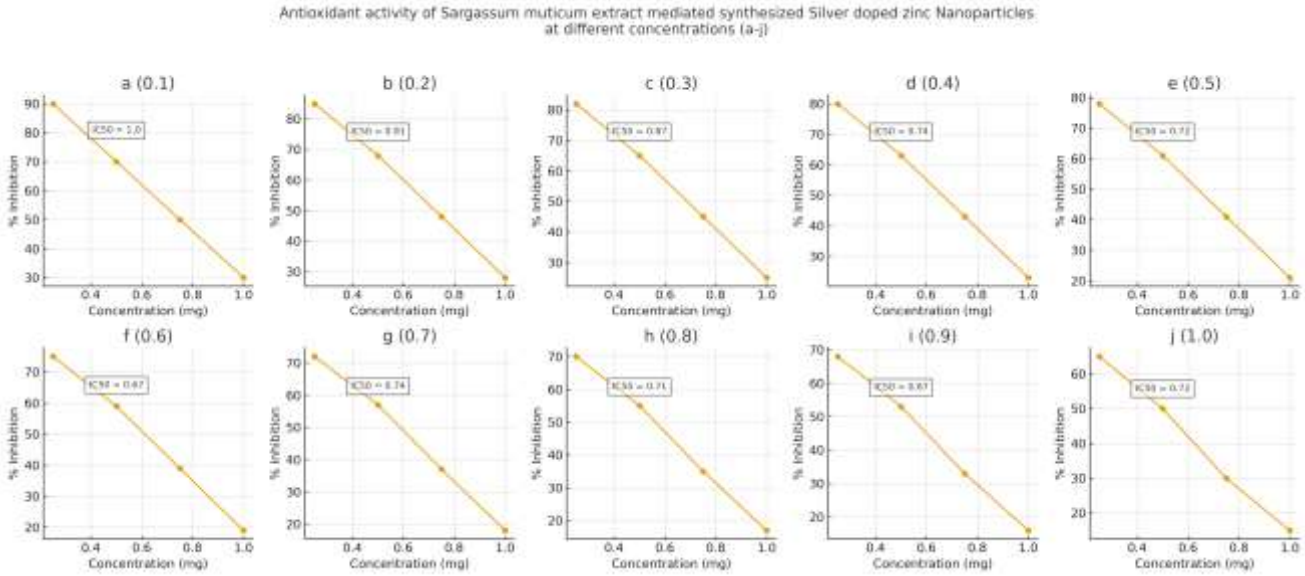

Figure 1 IC50 Values of Ag–ZnO Nanoparticles Synthesized using Sargassum muticum.

Table 3. Dose-dependent DPPH radical scavenging activity of Ag–ZnO nanoparticles synthesized from *Sargassum muticum* and *Gracilaria 58orticate*

Concentration (mg/ml)	Sargassum NPs – % Inhibition	Gracilaria NPs – % Inhibition
0.25	20	18
0.5	36	28
0.75	56	46
1.0	76	66

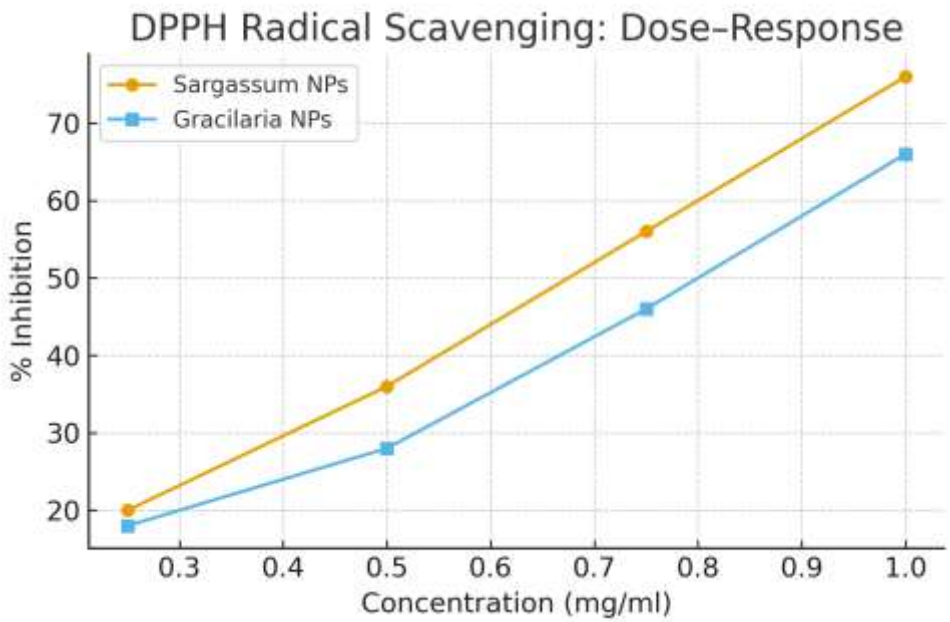

Figure2. Dose-dependent DPPH radical scavenging activity of Sargassum-derived Ag–ZnO nanoparticles compared with Gracilaria-derived nanoparticles.

4. Conclusion

This study confirms the successful green synthesis of silver-doped ZnO nanoparticles using Sargassum muticum. Structural characterization validated nanoparticle formation, while antioxidant assays demonstrated dose-dependent radical scavenging activity with an IC50 of ~0.67 mg/ml. The superior antioxidant potential compared to Gracilaria-derived nanoparticles highlights the biomedical promise of brown algae-mediated synthesis for sustainable nanotechnology applications.

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